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DRAWINGS ATTACHED

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(19) THE PATENT OFFICE LONDON

(54) METHOD FOR INDICATING AN ASSAY OF THE BACTERIOLOGICAL CONTENT OF A SAMPLE FLUID

(71) We, AEROJET - GENERAL CORPORATION, a Corporation duly organized and existing under the laws of the State of Ohio, United States of America, of 9100, East Flair Drive, El Monte, California, United States of America,

do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to the assay of the bacteriological content of a sample fluid.

The principal object of the invention is to provide a simple method for obtaining an indication of the relative amount of organic matter, such as bacteria, present in a general background of more or less similar matter.

The phenomenon of chemiluminescence has been used heretofore for the detection and quantitative measurement of biological organisms such as are encountered in samples of polluted air and water, biological warfare preparations, food contaminants, sterilization system products, etc. The chemiluminescence technique has been practiced by the production of light by reaction of luminol (Trade Mark) and hydrogen peroxide in the presence of these micro-organisms.

In accordance with the present invention, there is provided a method capable of detecting and indicating relative amounts of organisms, whether living or dead, and with relatively great sensitivity and, by use of this invention, continuous monitoring of the porphyrin content of various fluids may be accomplished.

The method of the invention thus comprises providing in a stream a predetermined quantity of the sample fluid; providing a stream of reagent capable of reacting with the bacteria in the sample fluid which reaction is characterised by the emission of light according to the bacteriological content of the sample fluid; mixing said streams in a cell to form a single stream exiting from the cell; sensing the light emitted from the cell; integrating the result of said sensing; and indicating the result of said integrating.

The following particular description of an embodiment of the invention is with reference to the accompanying drawings in which:

Fig. 1 is a block diagram of preferred equipment operative according to the method of performing the invention;

Fig. 2 illustrates graphically the generation of light output from a single static reaction;

Fig. 3 illustrates graphically the generation of light output from a continuous flow reaction as obtained from the equipment of Fig. 1; and

Fig. 4 illustrates graphically the effects of integrating the output represented in Fig. 3.

The method of the invention has been practiced very effectively with the system of Fig. 1 which shows it in block diagram form. In this drawings, reactor cell 10 is of a transparent material such as glass. Fluid containing the sample to be monitored is placed in sample container 11 from which conduit 12 is brought into cell 10. The reagent to be used, typically a mixture of luminol and hydrogen peroxide, is placed in container 13 from which conduit 14 is brought into cell 10. Conduit 15, leading from within cell 10, leads to valve 16 which is operable by arm 17 from solenoid 18. The output from valve 16 is carried through conduit 19 to trap 20. Conduit 22 connects trap 20 to vacuum source 23 which, when valve 16 is opened, causes fluid to be drawn from containers 11, 13 into and through cell 10, through valve 16 to trap 20. The fluid may comprise a liquid mixture or a liquid-gas mixture, the liquid of which will be diverted by trap 20 while the gas of which will enter vacuum source 23. This fluid system is effectively sealed from the atmosphere, excepting for reagent, sample and waste containers 13, 11 and 25 respectively, so that vacuum source 23 can provide a continuous flow of mixed

[*Price 25p*]

reagent and sample through cell 10. The liquid in trap 20 is released through conduit 24 to waste 25.

Cell 10 resides within light proof housing 26, shown partly broken away, and, in proximity to cell 10 within housing 26, there is located light-responsive device 27 such as a photo-mupltiplier tube. Since no light enters housing 26 from outside, tube 27 responds only to light from cell 10 and, of course, provides an output proportional to the intensity of light from cell 10. Power for the system is provided from source 28, ordinarily an a.-c. source, over line 28 to supply 29 which generates the high-voltage required by tube 27 connected to it over line 30. The output of tube 27 is carried over line 31 to the input of integration circuit 32 which integrates and amplifies. A suitable integrator is that illustrated and described in *Handbook of Operation Amplifier Applications*, First Edition, by Burr Brown Research Corp. 1963. The output of circuit 32 is brought over line 33 to read-out device 34, which may be a voltmeter, oscilloscope, etc.

Neither solenoid 18 nor circuit 32 nor read-out 34 become operable coincident with the application of power from source 28 to supply 29 when switch 28b is closed. Instead, the operation is programmed in that devices 18, 32 and 34 are made operable by energization through mechanism 36, driven by motor 37 whose shaft 38 is coupled to shaft 39 which carries cams 40, 41 and 42 associated with respective switches 43, 44, 45. Line 46 from line 28a brings power to motor 37 over line 47 and also to switches 43, 44, 45 over line 48 when switch 49 is closed. Line 50 from switch 43 energizes solenoid 18; line 51 from switch 44 energizes read-out 34; and line 52 from switch 45 energizes circuit 32.

After putting source 23 into operation and closing switch 28b, the system can be put through its cycle by closing switch 49, which will operate motor 37 to turn shaft 39. Solenoid 18, circuit 32 and read-out 34 will then be rendered operable at times and for periods established by the closure of switches 43, 44, 45, which will depend on the positions and contours of cams 40, 41, 42.

To enhance sensitivity, it has been found desirable to carry on the integration and read-out operations before, during and following the fluid flow through cell 10, for example, for about 30 seconds, during only about 10 seconds of which liquid is caused to flow. Generally, read-out 34 will be operated throughout the integration, but not necessarily. Considering an entire operation of about 30 seconds, switch 49 is closed to start motor 37. Then at a time T_o, switch 45 closes and renders circuit 32 operable to apply signal to read-out 34. About 10 seconds later, switch 43 closes and energizes solenoid 18, causing its arm to open valve 16 which will allow source 23 to draw liquid from containers 11 and 13 through cell 10 to trap 20. Fluid thus flows through cell 10 for about 10 seconds, whereupon switch 43 opens again. Throughout this time, switch 45 remains closed and stays closed for about 10 seconds after valve 16 has been shut. If cam 41 is arranged to coincide with the operation of cam 42, read-out 34 will be operative during the same 30 seconds, and thus it may be found more convenient to eliminate cam 41 and simply connect line 51 to switch 45.

It will be recognized that the foregoing time periods are given only by way of example and that others may be selected. These will usually depend on the material being sampled and the reagent being used; practical flow periods will generally range from about 5 to 30 seconds.

This method is capable of indicating relative quantities of porphyrins, ATP and also free radicals such as the —OH radical, organic radicals or others. This will include the detection and monitoring for bacteria or tissue cells inasmuch as porphyrins are associated with these materials.

The sample and the reagent begin to flow through cell 10 upon opening, at time T_o, of valve 16.

As indicated in Fig. 2, illumination from a single reaction, that is, in a non-flow system, does not occur instantaneously, but requires a finite period of time T_r. The total instantaneous emission from within cell 10, where the sample and reagent are being introduced continuously (Fig. 3) however, will initially rise from zero until, at time T_1, this emission will reach a steady state level. Similarly, the emisison will decay from the steady state level to zero in the time T_r upon closing of valve 16.

Photo-multiplier tube 27 senses some fraction of the total instantaneous photo energy emitted from within cell 10 and converts this energy into a proportional instantaneous electrical output signal. After amplification, this signal appears as a voltage, at the input to circuit 32, which is operable (Fig. 4) for the total sample period T_s and the output voltage at the termination of this sample period is expressed by

$$e = K \int_{T_o}^{T_o + T_r} E\,dt$$

Where

K=a system constant (contemplating quantum efficiency, amplifier gain, and flux distribution); and
E=luminescence intensity of the reaction.

This integrated voltage value is proportional to the total accumulated photon energy

emitted from within cell 10 over the sample period T_s.

It is noted that in the present continuous flow reaction method, the sample volume is not restricted to an upper limit. For a non-flow system, such a limit does exist and the volume of reaction cell 10, and therefore the sample, cannot be increased without limit because of optical coupling requirements. Furthermore, a continuous flow method lends itself to a much more uniform and reproducible mixing of the sample and reagent and, of course, continuous real-time assaying of a sample is possible.

WHAT WE CLAIM IS:—

1. A method for indicating an assay of the bacteriological content of a sample fluid, comprising: providing in a stream a predetermined quantity of the sample fluid; providing a stream of reagent capable of reacting with the bacteria in the sample fluid which reaction is characterized by the emission of light according to the bacteriological content of the sample fluid; mixing said streams in a cell to form a single stream exiting from the cell; sensing the light emitted from the cell; integrating the result of said sensing; and indicating the result of said integrating.

2. The method of claim 1 in which said sensing is photoelectric.

3. The method of claim 2 in which said photoelectric sensing includes photomultiplication.

4. The method of claims 1, 2 or 3 in which the output of said single stream is controlled by a valve.

5. The method of any of claims 1 to 4 in which the timing of the process is by operating motor-driven, cam-actuated switching.

6. The method of any of claims 1 through 5 in which said sensing is for a period extending from a time prior to a time subsequent to said mixing.

7. The method for indicating an assay of the bacteriological content of a sample of fluid substantially as hereinbefore described with reference to the accompanying drawings.

MARKS & CLERK,
Chartered Patent Agents,
57 & 58, Lincoln's Inn Fields,
London, WC2A 3LS.
Agents for the Applicant(s).

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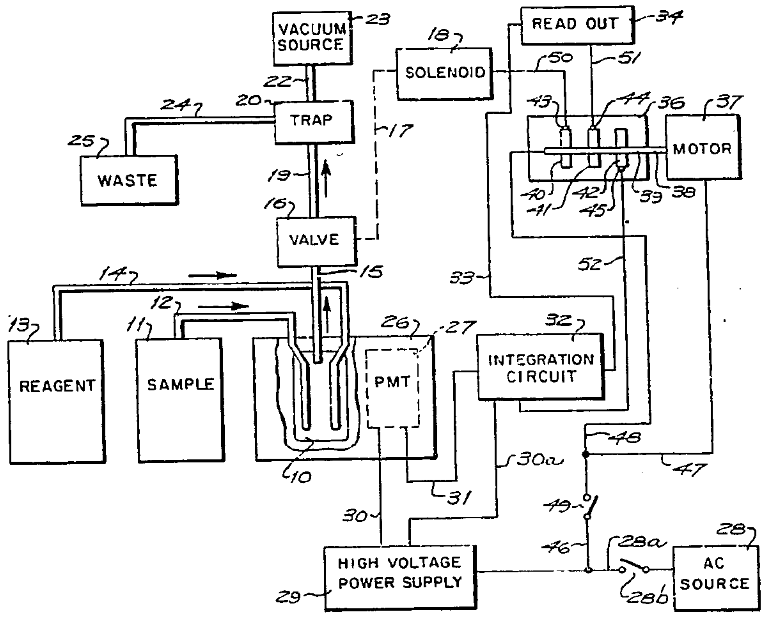
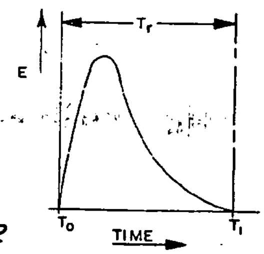

VACUUM SOURCE
23
18
SOLENOID
READ OUT
34
50
51
22
20
24
TRAP
17
43
44
36
37
MOTOR
25
WASTE
19
16
VALVE
40
41
42
45
39
38
14
15
33
52
12
13
11
26
27
32
REAGENT
SAMPLE
PMT
INTEGRATION CIRCUIT
48
10
30a
47
31
30
49
46
28a
28
HIGH VOLTAGE POWER SUPPLY
AC SOURCE
29
28b

FIG. 1

131546 COMPLETE SPECIFICATION

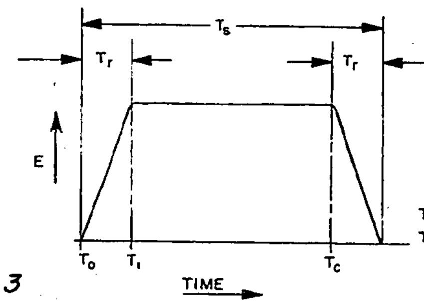
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 2



OUTPUT FROM A SINGLE REACTION UNIT

FIG. 2

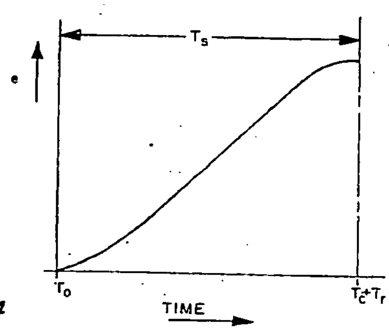


OUTPUT FROM A CONTINUOUS FLOW REACTION

T_o = opening of vacuum valve

T_c = closing of vacuum valve

FIG. 3



INTEGRATED SIGNAL OF CONTINUOUS FLOW SYSTEM

$$e = K \int_{T_o}^{T_c + T_r} E\,dt$$

FIG. 4

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